

EXHIBIT 2

APPLICATION
FOR CONTINUATION GRANT

ALY	5	123	AL2028-02
TOTAL PROJECT PERIOD			
From:		Through:	
REQUESTED BUDGET PERIOD			
From:		Through:	

To Be Verified By Applicant. Check Information in Items 1 Through 6. If Incorrect, Furnish Correct Information in Item 13.

1. TITLE

CDNA ANALYSIS FOR SUBSET-SPECIFIC T-CELL GENE EXPRESSION

2a. PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR
(name and address, street, city, state, zip code)

KWON, BYCUNG S
GUTHRIE FDN FOR MEDICAL RES
GUTHRIE SQUARE
SAYRE, PA 18840

4. APPLICANT ORGANIZATION (name and address, street, city, state, zip code)

GUTHRIE FDN FOR MEDICAL RESEARCH
GUTHRIE SQUARE
SAYRE, PA 18840

5. ENTITY IDENTIFICATION NUMBER

124602295/A1

2b. DEPARTMENT, SERVICE, LABORATORY OR EQUIVALENT

MOLECULAR GENETICS LABORATORY

2c. MAJOR SUBDIVISION

3. ORGANIZATIONAL COMPONENT TO RECEIVE CREDIT FOR
BIOMEDICAL RESEARCH SUPPORT GRANT (see instructions)

6. TITLE AND ADDRESS OF OFFICIAL IN BUSINESS OFFICE
OF APPLICANT ORGANIZATION

TREASURER
GUTHRIE FDN FOR MEDICAL RESEARCH
GUTHRIE SQUARE
SAYRE, PA 18840

60 OTHER RESEARCH ORGANIZATION

COMPLETE THE FOLLOWING (See Instructions)

7. HUMAN SUBJECTS

☒ NO ☐ YES { OR Exemption # _____
☐ Form HHS 596 enclosed

8. RECOMBINANT DNA

☐ NO ☒ YES

9. PERFORMANCE SITE(S) (organizations and addresses)

Molecular Genetics Laboratory
Guthrie Foundation for Medical Research
Guthrie Square
Sayre, PA 18840-1692

11. INVENTIONS (see instructions)

☒ NO ☐ YES { ☐ Previously reported
☐ Not previously reported

TELEPHONE INFORMATION

	AREA CODE	TELEPHONE NO. AND EXTENSION
12a. PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR (Item 2a)	717	888-6666, X4632
12b. NAME OF BUSINESS OFFICIAL (Item 6)	717	888-6666, X4620
12c. NAME AND TITLE OF OFFICIAL SIGNING FOR APPLICANT ORGANIZATION (Item 15)	717	888-6666, X4620
John M. Thomas, M.D.	717	888-6666, X4620

10. DIRECT COSTS REQUESTED FOR BUDGET PERIOD

\$35,792

13. USE THIS SPACE FOR CORRECTIONS TO ITEMS 1 THROUGH 6. INDICATE THE NUMBER(S) WHERE ANSWER(S) APPLY.

14. PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR ASSURANCE: I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application. Willful provision of false information is a criminal offense (U.S. Code, Title 18, Section 1001).

SIGNATURE OF PERSON NAMED IN 2a. (In ink. "Per" signature not acceptable)

DATE

15. CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true and complete to the best of my knowledge, and accept the obligation to comply with the Public Health Service terms and conditions if a grant is awarded as the result of this application. A willfully false certification is a criminal offense (U.S. Code, Title 18, Section 1001).

SIGNATURE OF PERSON NAMED IN 12c. (In ink. "Per" signature not acceptable)

DATE

SECTION I (continued)
SUMMARY OF PROPOSED WORK

GRANT NUMBER

1R23A123058-01

KEY PROFESSIONAL PERSONNEL ENGAGED ON PROJECT

NAME	POSITION TITLE	DEPARTMENT AND ORGANIZATION
Byoung S. Kwon	Assistant Scientist Head, Molecular Genetics Lab.	Guthrie Research Institute
Gwan Shik Kim	Research Associate	Guthrie Research Institute

Give a brief summary of plans for the next year of support, including the objectives and specific aims as well as the methodology to be used to achieve these aims. **DO NOT EXCEED THE SPACE PROVIDED.**

Utilizing the approach proposed in the original proposal, we isolated 16 cDNA clones; 9 from T helper (Th) and 7 from cytolytic (CTL) T cells. Each clone was T cell specific and expressed preferentially in Th or CTL. One from Th corresponded to pro-opiomelanocortin and one from CTL corresponded to serine esterase-like molecule. The other 14 cDNA sequences have not been previously reported. In the next year our primary objective will be to characterize and identify the molecules corresponding to the 14 cDNA inserts. Specific aims include isolation of full length cDNA inserts for each of the 14 cDNAs, determination of the nucleotide sequence, and elucidation of the primary structure of the proteins. We will rescreen Th (L_2) and CTL (L_2) cDNA libraries which we have earlier prepared in λ gt10 and λ gt11 vectors, using each of the cDNA inserts as a probe. The cDNA inserts whose sizes are similar to that of corresponding mRNA will be selected. The nucleotide sequence of the full length cDNA inserts for each of the clones will be determined by dideoxy chain termination method after cloning into M13 vectors. The deduced amino acid sequence will be determined and characterized as to whether the molecules are secretory or nonsecretory. This will help predict their more exact roles in helper or cytolytic T-cell activities.

VERTEBRATE ANIMALS INVOLVED ☒ NO ☐ YES If "YES," identify by common names and underline primates.

SECTION II NEXT BUDGET PERIOD Follow instructions carefully		FROM [REDACTED]	THROUGH [REDACTED]	GRANT NUMBER 1R23A123058-01		
A. ITEMIZE DIRECT COSTS REQUESTED FOR NEXT BUDGET PERIOD				DOLLAR AMOUNT REQUESTED (Omit cents)		
PERSONNEL (Applicant organization only) (See instructions)						
NAME	TITLE OF POSITION	TIME/EFFORT		SALARY	FRINGE BENEFITS	TOTALS
		%	Hours per Week			
Byoung S. Kwon	Principal Investigator	50	20	16,000	2,992	
Lynn Usack	Lab Technician	100	40	14,000	2,800	
SUBTOTALS				30,000	5,792	35,792
CONSULTANT COSTS (See instructions)						
NONE						
EQUIPMENT (Itemize)						
NONE						
SUPPLIES (Itemize by category)						
NONE						
TRAVEL						
DOMESTIC						
FOREIGN						
PATIENT CARE COSTS						
INPATIENT NONE						
OUTPATIENT NONE						
ALTERATIONS AND RENOVATIONS (Itemize by category)						
NONE						
CONSORTIUM/CONTRACTUAL COSTS (See instructions)						
NONE						
OTHER EXPENSES (Itemize by category)						
NONE						
TOTAL DIRECT COST (Enter on Page 1, Item 10)						35,792
INDIRECT COST (See instructions)	44 % S&W*	*If this is a special rate (e.g. off-site) explain		Date of DHHS agreement	<input type="checkbox"/> Not requested <input type="checkbox"/> Under negotiation with:	
	44 % TDC*					

SECTION III CURRENT BUDGET PERIOD	FROM [REDACTED]	THROUGH [REDACTED]	GRANT NUMBER 1R23AI23058-01
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The following pertains to your CURRENT PHS budget. Do not include cost sharing funds. This information in conjunction with that provided on Page 2 will be used in determining the amount of support for the NEXT budget period.

A. BUDGET	CURRENT BUDGET (as approved by awarding unit)	ACTUAL EXPENDITURES THRU (insert date): [REDACTED]	ESTIMATED ADDITIONAL EXPENDITURES AND OBLIGATIONS FOR REMAINDER OF CURRENT BUDGET PERIOD	TOTAL ESTIMATED EXPENDITURES AND OBLIGATIONS (Col. 2 plus Col. 3)	ESTIMATED UNOBLIGATED BALANCE (Subtract Col. 4 from Col. 1)
	(1)	(2)	(3)	(4)	(5)
TOTAL DIRECT COSTS	34,304	22,800	11,504	34,304	-0-
INDIRECT COSTS (as provided)	15,094	10,131	4,963	15,094	-0-
TOTALS →	49,398	32,931	16,467	49,398	

B. THROUGH F.

See instructions and provide the information required in items B. through F. Use this page and continuation pages as necessary.

B.	NAME	TITLE	CATEGORY	LESS THAN 25%	26-50%	51-75%	MO/ TH/
	Byoung Kwon	Assistant Scientist	1		*		
	Gwan Kim	Research Associate	2	X			

C. NONE

D. 6th International Congress on Immunology, Toronto, Canada [REDACTED]

F. Feasibility Grant from the American Diabetes Association, Inc.

SECTION IV PROGRESS REPORT SUMMARY		GRANT NUMBER 1R23A123058-01	
PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR Byoung Kwon		PERIOD COVERED BY THIS REPORT	
NAME OF ORGANIZATION Guthrie Foundation for Medical Research		FROM [REDACTED]	THROUGH [REDACTED]
TITLE (Repeat title shown in item 1 on first page) cDNA Analysis for Subset-Specific T-cell Gene Expression (SEE INSTRUCTIONS)			

- No change.
- cDNA libraries were prepared from cloned murine helper (Th) and cytolytic (CTL) T lymphocytes. Both negative and positive differential screening and RNA blot analysis were used to identify clones which were T-cell specific and expressed preferentially in Th or CTL. Seven clones corresponded to previously described T cell genes, and 16 additional cDNA clones were isolated, 9 from Th and 7 from CTL cells. Of these clones 3 were expressed in both Th and CTL, 7 were expressed in only Th and 6 only in CTL. The 16 cDNA inserts were cloned into M13 vector mp8 and their partial nucleotide sequence was determined. The nucleotide sequence of each cDNA was compared with sequences in the Gene Bank and with cDNA sequences from CTL or Th which have been published recently. One gene from CTL was homologous to a serine esterase-like sequence (Gershenfeld and Weissman, Science 232:854, 1986) and one gene from Th proved to be identical to pro-opiomelanocortin. The other 14 cDNA sequences were not previously reported. Next, these clones were analyzed for induction by IL-2 or T cell antigen receptor stimulation. Three different patterns of expression were documented: 1) inducible only by ConA; 2) inducible by ConA and IL-2; and 3) inducible by ConA and T cell antigen receptor stimulation. The detailed work is described in an accompanying manuscript entitled, "Isolation and initial characterization of multiple species of T lymphocyte subset cDNA clones". The protocol for differential screening of a cDNA library which we developed will be generally applicable to a situation where a preselected cDNA library is undesirable. Characterization of Th- or CTL-specific cDNA clones, which we have isolated, will lead to the discovery of as yet unknown roles for T cell subsets in the immune responses and to better definition of the pathways of T-cell activation process.

- To isolate full length cDNA inserts for each of the 14 novel cDNAs which are T cell-specific and expressed preferentially in Th or CTL.
- To determine entire nucleotide sequence of as many as possible of the full length cDNA inserts.
- To demonstrate the primary structure of the proteins corresponding to the novel cDNA inserts.

PUBLICATIONS

- Byoung S. Kwon, Gwan S. Kim, Michael B. Prystowsky, David W. Lancki, Daniel E. Sabath, Julian Pan and Sherman Weissman. Isolation and initial characterization of multiple species of T lymphocyte subset cDNA clones. Submitted to Proc. Natl. Acad. Sci. USA.
- Byoung S. Kwon, Asifa K. Haq, Seymour H. Pomerantz and Ruth Halaban. Isolation and sequence of a cDNA clone for human tyrosinase which maps at the mouse albino locus. Submitted to J. Biol. Chem.